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The kinetics of bulk, in-situ, hydration of the disaccharides α -lactose and trehalose by neutron powder diffraction.

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Introduction

Many saccharides, including di-, tri- and poly-saccharide forms and their hydrated derivatives, are important excipients.¹ As a result understanding the hydration behaviours of these saccharides is highly relevant in their application as filler-binders in tablets and solid form inhalers where hydration and dissolution factors control tablet and particle disintegration rates. Lactose, β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose, is a disaccharide that consists of galactose and glucose fragments bonded through a β -1 \rightarrow 4 glycosidic linkage; in α -lactose the glucose is in the α -pyranose form.² α -Lactose is also known as a monohydrate, whose crystal structure has recently been redetermined.³ Lactose and modified lactoses are widely used as filler-binders in the formation of tablets by direct compression so controlling the properties of these materials in relation to their compaction and dissolution behaviours is an area of considerable current interest. For example, recent work has shown that modified anhydrous α -lactose with high surface areas have improved compaction and dissolution behaviours.^{4,5} Trehalose, α -D-glucopyranosyl-(1 \rightarrow 1)- α -D-glucopyranoside, is formed from two glucose units joined by a 1-1 alpha bond.^{6,7} it frequently crystallizes as the dihydrate.^{8,9} Trehalose is used as a protein-stabilizing agent and is used in several biopharmaceutical monoclonal antibody formulations, in organ protection solutions and as a moisturizer in cosmetics and dry eye treatments. It is also described as a potential excipient for use in powder inhalers.

The importance of studying the behaviours of (bio)pharmaceutical compounds, particularly *in situ* during thermal or humidity changes, has been recently discussed by a number of authors.¹⁰⁻¹³ Information on phase stability and changes as a result of heating or cooling or relative humidity can be obtained using powder X-ray diffraction, including structural changes that occur as a result of water or solvent molecule uptake. However, the powder X-ray diffraction method has drawbacks as it samples mainly the surface of a polycrystalline mixture so that it is phase changes occurring on the surface of a crystallite that are probed. This limitation is of particular concern when investigating the phase and reaction chemistry of a pharma-compound as a function of particle size – a key parameter in controlling dissolution rates.^{14,15} Similar limitations apply to other commonly used techniques for studying pharmaceuticals *in situ*, such as infrared and Raman spectroscopy and optical microscopy, in that these methods also monitor the surface

reaction chemistry. Therefore to determine changes in bulk and overall structure, and also kinetics as a function of particle size, a method that probes the complete sample is required. To this end we have used neutron powder diffraction (NPD), which is well known as a non-destructive technique for *in situ* investigations of bulk systems and non-surface structures, for example archaeological artefacts and engineering structures. Problems with incoherent scattering, which may have precluded application of this method to hydrogenous pharmaceutical compounds previously, have recently been overcome by the use of high flux instrumentation. Here, we describe the *in situ* hydration of α -lactose, of two particle sizes, and trehalose followed by NPD, thereby providing information on the kinetics of these hydration processes for the bulk polycrystalline material.

Experimental

Neutron diffraction experiments were performed at the ILL in Grenoble, using the high-flux powder diffraction instrument D20, operating in its high take-off angle configuration at a fixed wavelength of 1.86 Å.¹⁶ High quality powder diffraction data were initially obtained from anhydrous α -lactose (>99.9%, particle size ~50 μ m, hereafter denoted HC α -lactose) and its monohydrate (>99.9%); samples were mounted in 7mm diameter vanadium cans (~3g) and a data were collected over a period of 4 hours in each case. After normalisation and reduction data were analysed *via* Rietveld refinement using the GSAS¹⁷ in conjunction with the EXPGUI¹⁸ user interface using the published crystallographic models.^{2,3}

For *in situ* hydration experiments powdered samples of α -lactose (two samples, the high crystallinity form described above and a small particle size form (particle size 0.1-1 μ m), hereafter denoted SPS α -lactose) or trehalose (>99%) were loaded into 6 mm diameter container formed from a heavily perforated vanadium-foil. The can size was kept to a minimum to reduce the possibility of moisture gradients between powder at the surface and the core of the can while ensuring that diffraction data collected in 2.5 minute intervals were of sufficient quality to permit profile refinement with extraction of phase fractions. The sample environment consisted of heated aluminium vessel with an atmosphere of controlled-humidity modified from that described previously.¹⁹ A regulated flow of humid gas was achieved through the use of a RH-200 humidity generator unit, which combines a stream of dry nitrogen gas with a humidified N₂ stream in a mixing ratio that can be set and remotely controlled *via* a computer program. The

humidity of the output gas stream was directly measured within the generator unit using a dew-point sensor and in the sample chamber using a portable hygrometer. Temperature control was achieved over the temperature range 30-200°C through heating tapes mounted around the aluminium chamber and a remote PID interface; pre-experiment tests showed that 100% humidity could be achieved up to 55°C and lower humidity values at the higher temperatures. The background contribution from the humid gas flow through the sample environment was found to be negligible by measurements on a non-hygroscopic powder at various humidity levels, dispelling concerns of significant beam attenuation or incoherent scattering by the H₂O vapour.

The reaction progress of hydration reactions in a neutron beam can be monitored through the increase in total counts scattered into the detectors which increases monotonically with level of hydrogen in the sample. Full powder diffraction patterns were collected at 2.5 min intervals during the hydration of HCa-lactose, SPSα-lactose and trehalose. Diffraction profiles were modelled *via* Rietveld refinement as stated above; for the trehalose pairs of diffraction data sets were summed to give the equivalent of 5 minute data sets. Analysis was undertaken using the published crystallographic models for α-lactose, α-lactose monohydrate, trehalose and trehalose dihydrate.^{2,3,6,8} Crystallographic parameters were not refined; only the profile parameters and the phase fractions of the anhydrous and hydrated disaccharides; sequential GSAS refinements (SEQGSAS)¹⁶ was used to analyse the full set of diffraction profiles for each system and the phase fraction ratio was extracted.

Results

Figure 1 shows the diffraction profiles for HCa-lactose collected as a function of time; strong peaks of the monohydrate phase that occur between 49 and 52° start to appear after 30 minutes and become dominant after ~120 minutes. For SPSα-lactose the appearance of the monohydrate was significant faster; this is evident in the extracted phase percentages of the monohydrate in the sample as a function of time as plotted in Figure 2 for the two different particle sizes. The rate of growth of the monohydrate phase for HCa-lactose is approximately twice as fast as for SPSα-lactose. The Avrami equation can be used to describe transformation between two solid crystalline phases and also provide some information on the likely mechanism involved.²⁰ This expression

$$X = 1 - e^{-(k[t])^n}$$

Where X is the fraction of transformed phase (in this case the hydrated disaccharide), t the elapsed time, k a rate constant and n the order of the reaction. Therefore using the derived expression

$$\ln[-\ln(1 - X)] = n \ln k + n \ln t$$

values of n can be extracted from appropriate plots. Figure 3 shows the appropriate ln-ln plots for the two α -lactose samples and these data are well fitted by a straight line in accordance with a single phase transformation and the Avrami equation. Extracted values of n are: HC α -lactose 1.67 ($r^2 = 0.995$) and SPS α -lactose 1.61 ($r^2 = 0.992$). Values of n reflect the dimensionality of the transformation or, for a diffusion controlled process, its nature; n may also be determined by the nucleation mode, for example site saturated mechanism versus continuous nucleation one. The values of n determined here, between 1 and 2, are consistent with diffusion controlled growth or, as is likely in this case nucleation and growth of a phase from a surface. Thus the hydrated phase seems to nucleate on the surface of the α -lactose crystallites in contact with the moist air and this phase then grows inwards from the surface. For the smaller SPS α -lactose particles this process moves to completion more rapidly in terms of the phase fraction of monohydrate due to the much smaller initial smaller particle size. This process is akin to the dissolution of poorly soluble pharmaceuticals where similar enhancements in rates are found with small particle sizes.^{13,14} This behaviour contrasts with that seen in amorphous lactose (a mixed product consisting of ca. 30% monohydrate and 70% anhydrous α -lactose) where growth rates of additional monohydrate appear to be limited by surface incorporation rather than diffusion, and in the absence of seed crystals nucleation appears to occur through a site-saturated mechanism.⁵

For trehalose a similar analysis shows slightly more complex behaviour, Figure 5. A straight line fit to the Avrami plot is much poorer ($r^2 = 0.984$) though it yields a similar value for n (1.645) as is found for lactose. A better fit to this plot can be achieved using two straight lines corresponding to a reaction time up to 150 minutes ($n = 2.35$, $r^2 = 0.973$) and thereafter ($n = 1.45$, $r^2 = 0.995$). This behaviour might be explained by the fact that the hydration of trehalose process leads eventually to trehalose dihydrate and probably involves a two stage mechanism with an

intermediate partially hydrated phase. As no additional unfitted reflection was observed at intermediate hydration times it seems likely that only very small levels of this intermediate phase are formed at the reaction boundary or it is amorphous. The values of n for these processes, between 2-3 and 1-2 respectively, are consistent with an initial 3-dimensional bulk growth process for the formation of any initial intermediate phase followed by a lower dimensionality, surface or linear, in the crystallisation of the dihydrate.

Conclusions

Neutron powder diffraction can be used to observe the bulk hydration or salvation of pharmaceutical compounds *in situ*. Information on the rates of such processes and their mechanisms can also be gleaned. For α -lactose a simple hydration process occurs which is consistent with the simple growth of dihydrate from the crystallite surface and this process occurs more rapidly to completion for smaller crystallite sizes. In trehalose a more complex mechanism seems to be involved in the formation of an intermediate hydrated phase on route to its dihydrate.

The ability to observe pharmaceutical compounds *in situ* in controlled humidity and temperature conditions and study bulk material rather than surfaces is of considerable relevance to production and the storage of many active pharmaceutical compounds. The technique could also readily be applied to complex formulations and those in pelletized forms.

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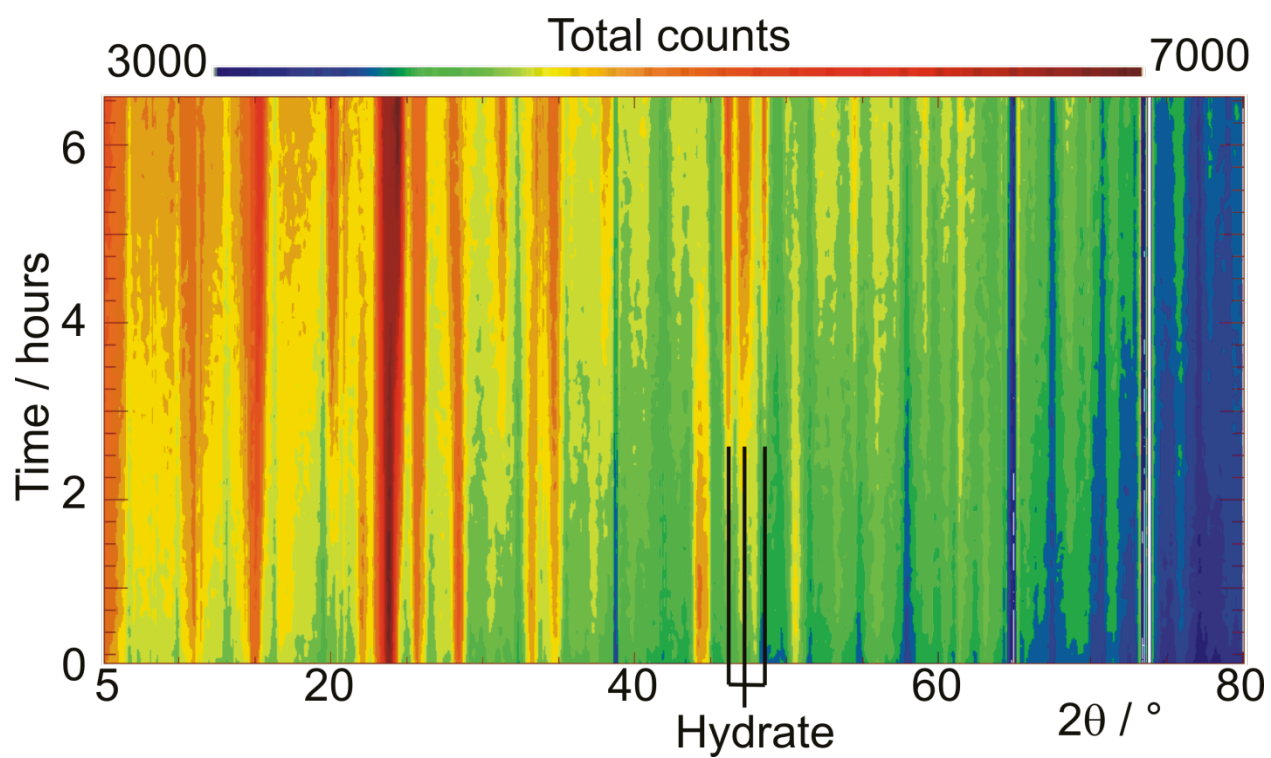


Figure 1. Stacked plot viewed down the intensity direction showing the evolution of the diffraction patterns of standard α -lactose as a function of time in 100% relative humidity at 45°C; the positions of three strong reflections from α -lactose monohydrate are shown

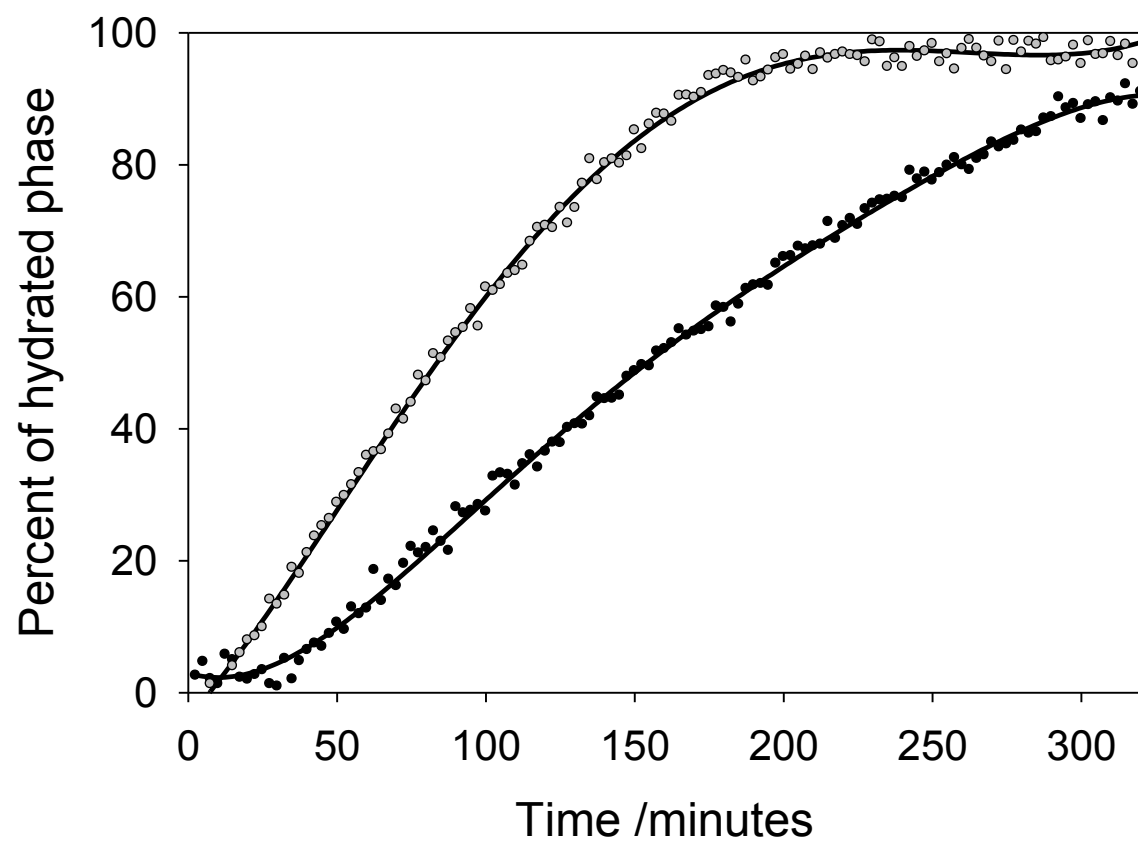


Figure 2. Phase percentage of α -lactose monohydrate in HCa-lactose (black filled circles) and SPS α -lactose (grey filled circles) as a function of time.

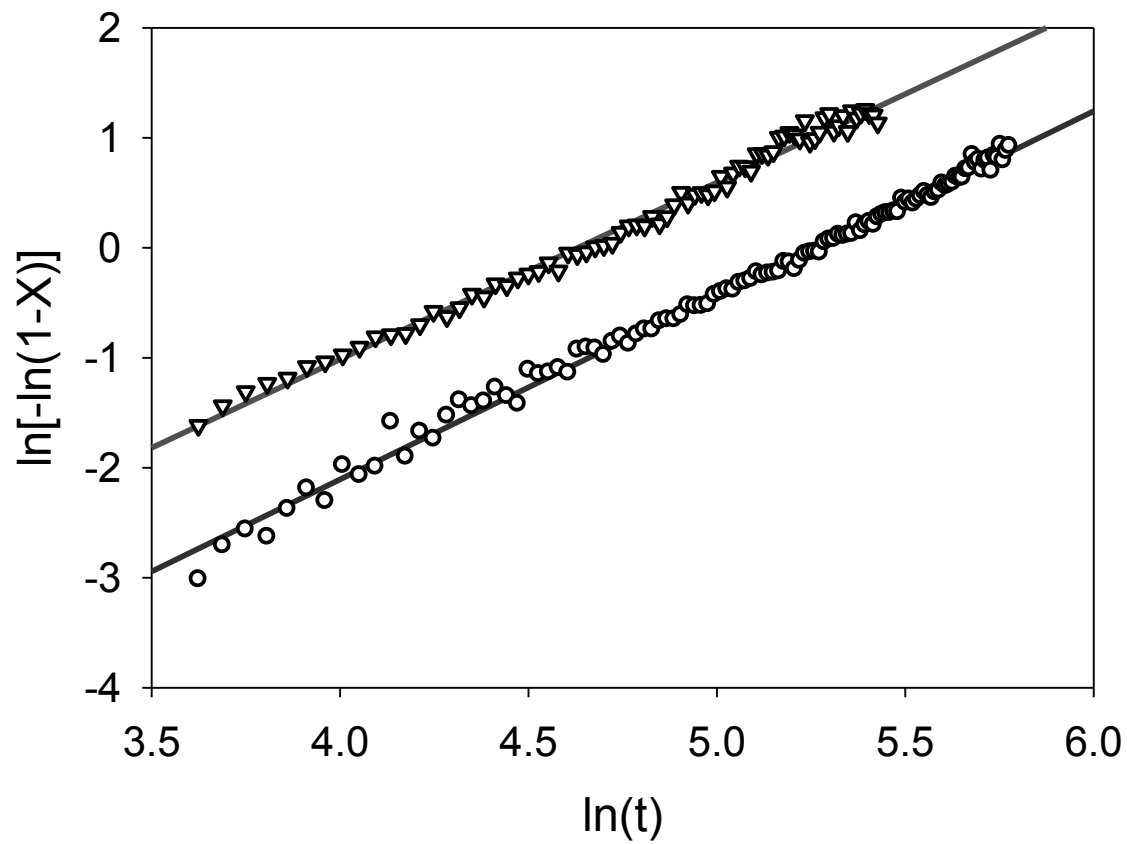


Figure 3. Avrami plots for the formation of α -lactose monohydrate in HCa α -lactose (circles) and SPS α -lactose (triangles) as a function of time

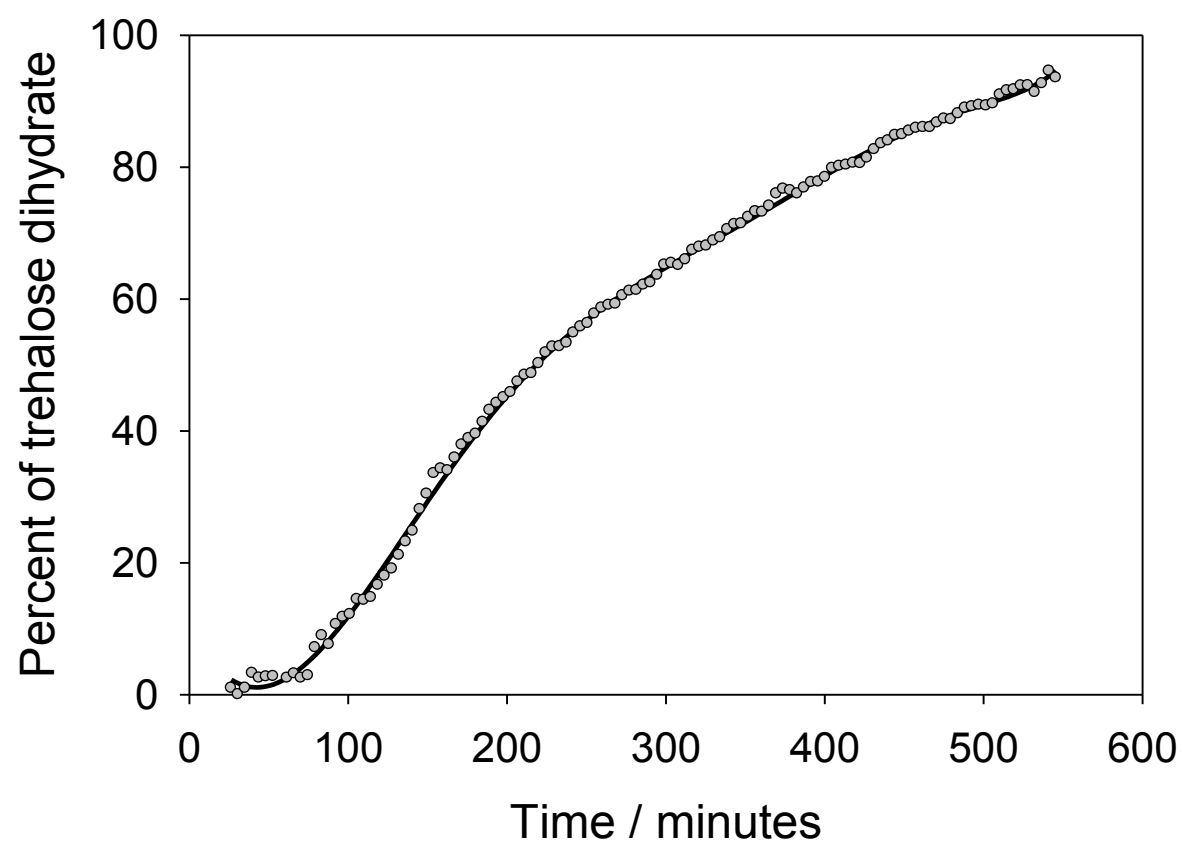


Figure 4. Phase percent of trehalose dihydrate as a function of time

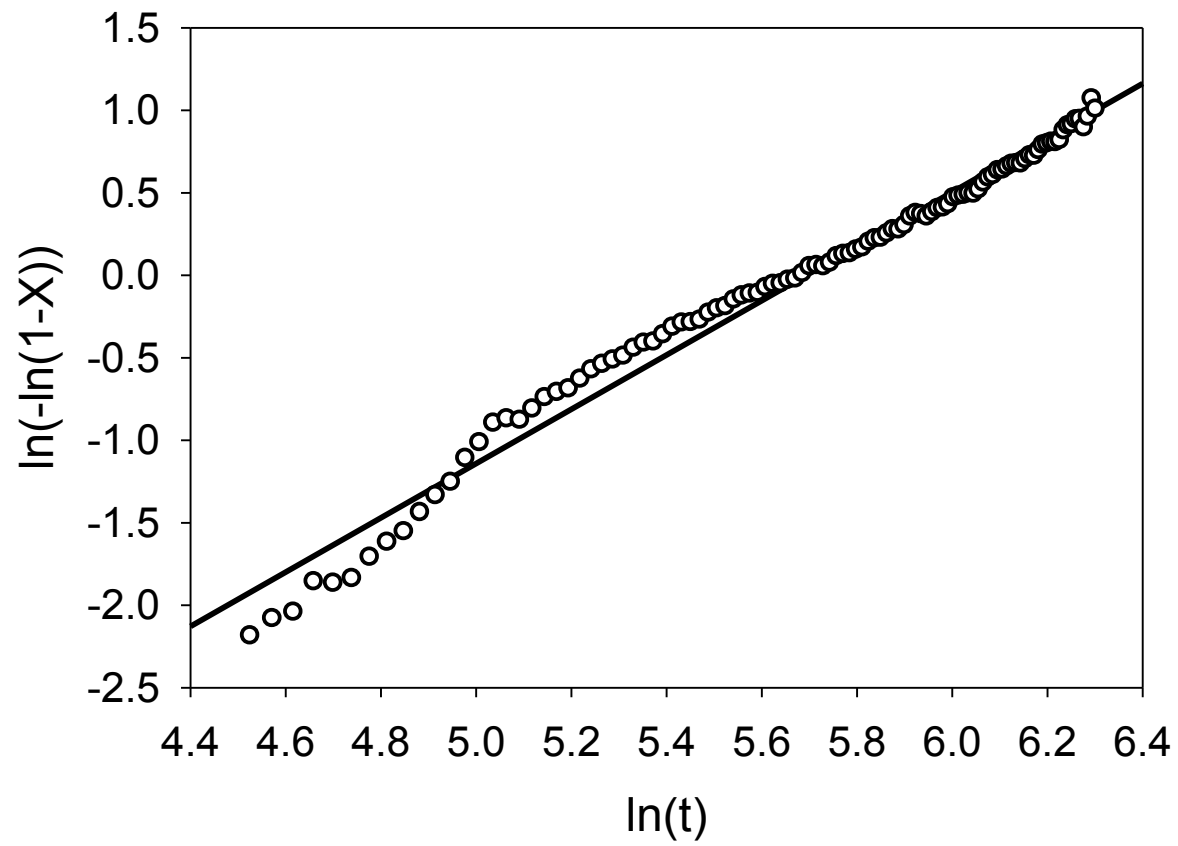


Figure 5. Avrami plot for the hydration of trehalose; linear regression fitted to whole data range.